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14. ABSTRACT
The goals of this initial year of funding were to develop engineered antibody fragments (cys-diabodies and cys-minibodies) suitable for labeling with positron-emitting radionuclides and fluorescent dyes for multimodal imaging of prostate cancer. A novel cys-minibody recognizing PSCA (prostate stem cell antigen) has been produced. Conjugation conditions have been developed for radiolabeling with either and I-124 and Zr-89 via lysine (random) or cysteine (site-specific) residues of the proteins. Targeting, imaging, and biodistribution studies in mouse models of prostate cancer confirm high activity in PSCA-positive tumors with excellent visualization by PET imaging. Recently, dual-labeling (with radionuclide and fluorescent dye) of the cys-minibody has been developed and evaluated in mouse models.

15. SUBJECT TERMS
Prostate cancer, imaging, antibody fragment, Positron emission tomography, fluorescence imaging, PSCA

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1. INTRODUCTION

Imaging remains a major unmet need in the management of prostate cancer. We are developing imaging probes based on engineered antibodies that recognize PSCA (prostate stem cell antigen), a cell surface protein highly expressed in prostate cancer. These engineered antibody fragments (cys-minibodies and cys-diabodies) can be labeled with radioisotopes for non-invasive PET imaging for use at multiple points in the prostate cancer treatment continuum, including staging at diagnosis, monitoring treatment, and re-staging at various points during management. Engineered fragments can also be labeled with fluorescent dyes for visual guidance in an intraoperative setting to ensure complete resection with negative margins. In this project, dually-labeled PSCA imaging agents are being developed that can be used for pre- and intra-operative detection of prostate cancer.

2. KEYWORDS

Prostate cancer, imaging, antibody fragment, positron emission tomography, fluorescence imaging, PSCA

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1. Develop universal optimized cys-diabody and cys-minibody fragments against PSCA for PET imaging of prostate and pancreatic cancer.
Subtasks:

Major Task 1. Develop and evaluate cys-diabody and cys-minibody fragments
Major Task 2. Design, optimize and test multifunctional, F-18, and alternatively labeled fragments
Major Task 3. New technologies: alternative site-specific labeling methods, use of click chemistry

Specific Aim 2. Evaluate the ability of lead PSCA fragments to imaging prostate cancer in disease progression in xenograft and genetically engineered models of prostate cancer
Subtasks:

Major Task 4. Image bone and lymph node in xenograft models
Major Task 5. Image transgenic mouse models
Major Task 6. Development and evaluation of singly labeled and optimized optical probes for surgery
Major Task 7. Development of dual labeled probes for PET and optical imaging.

For Dr. Wu (Partnering PI) during Year 1 of the project, the subtasks were:
Major Task 1

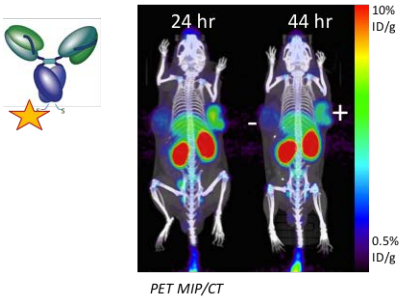
Subtask 1. Produce and purify A2 cys-diabody and A2-cys-minibody in mg quantities (months 1-6) **Completed.**

Subtask 2. Optimize radiolabeling conditions with I-124 (tyrosine) and Zr-89 (DFO conjugation to lysine and cysteine residues). Confirm retention of binding by QCM and cell binding. (months 3-9) **Completed.**

Subtask 3. Conduct microPET imaging and biodistribution in subcutaneous models using I-124 and/or Zr-89; provide PET tracers to Aim 2. (months 6-18) **Completed and ongoing.**

What was accomplished under these goals?

Year 1 has focused on the development, production, and optimization of the PET imaging agents. Two closely-related sequence variants, A2 and A11 have been evaluated. Of note, a novel A11 cys-minibody has been designed, produced, and purified, and this has been designated the lead candidate for singly and dually-labeled imaging agents. The protein has been radiolabeled with Zr-89 (site-specific) (**Figure 1**) and I-124 (**Figure 2**) for PET imaging studies.



	% ID/g ± SD
22Rv1 x PSCA	4.65 ± 2.02
22Rv1	2.57 ± 0.74
Blood	0.33 ± 0.05
Heart	2.67 ± 0.24
Lungs	1.49 ± 0.09
Liver	7.74 ± 0.91
Kidney	30.9 ± 2.52
Spleen	7.55 ± 2.14
Stomach	0.35 ± 0.05
Intestine	1.17 ± 0.10
Bone	5.46 ± 0.85
Muscle	0.31 ± 0.04
Pos:Neg Tum	2.54 ± 0.44
Tum:Blood	16.6 ± 3.67
Tum:Muscle	19.0 ± 4.18

n = 3

Figure 1. Imaging and biodistribution of A11 cys-minibody site-specifically conjugated using maleimide DFO and radiolabeled with 89Zr. Left, diagram of cys-minibody with C-terminal thiols and radionuclide. Center, immunoPET imaging of PSCA(-) and PSCA (+) tumors at 24 and 44 h post-injection showing antigen-driven localization and renal clearance. Right, biodistribution.

Importantly, significant progress has been made ahead of schedule on production and evaluation of a dually-labeled PET/optical probe, as detailed in the abstract below, which was presented at the Antibody Technology Resource Center Symposium at UC San Francisco, October 2016.

Dual-modality immuno-PET/fluorescence imaging of prostate cancer using anti-PSCA A11 cys-minibody

Wen-Ting Tsai[1], Kirstin Zettlitz[1], Richard Tavaré[1], Felix Salazar[1], Robert Reiter[2], and Anna Wu[1]
[1]Crump Institute for Molecular Imaging, Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA
[2]Department of Urology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Prostate cancer can benefit from non-invasive and more accurate diagnosis, as well as improved visualization during surgery. Immuno-PET can provide information on extent and location of the disease, while fluorescent image-guided surgery can distinguish cancerous tissue from healthy surrounding tissues for clean resection. Prostate Stem Cell Antigen (PSCA) is upregulated in the majority of prostate cancers and metastases and is therefore a promising target for imaging (Knowles *J Nucl Med* 55:429, 2014). Engineered antibody fragments, such as the minibody, exhibit ideal immuno-PET imaging characteristics due to fast blood clearance for high target-to-background images at short imaging times post-injection (Wu *Methods* 65:139, 2014). A dual-labeled minibody probe can reveal the current PSCA-expressing tumor burden by PET, while also identifying margins of malignancy by near-infrared fluorescence.
The humanized anti-PSCA A11 minibody (A11 Mb) was previously affinity matured by yeast scFv display (Lepin Eur. J. Nucl. Med. 37:1529, 2010), then engineered with a C-terminal cys-tag (A11 cMb) that can be site-specifically labeled by thiol-chemistry. ^{124}I-A11 cMb, ^{124}I-Cy5.5-A11 cMb, ^{89}Zr-DFO-A11 cMb, and ^{89}Zr-DFO-A11 cMb-Cy5.5 were used to image 22Rv1 tumors expressing PSCA. For dual-modality imaging, A11 cMb was site-specifically conjugated with Cy5.5-maleimide and radiolabeled with ^{124}I or ^{89}Zr. PET imaging with ^{124}I-Cy5.5-A11 cMb in nude mice bearing

subcutaneous PSCA-positive and negative tumors resulted in a positive-to-negative tumor ratio of 13:1 at 22 hours post-injection, comparable to the 8:1 ratio when imaged with ^{124}I-A11 cMb. The PSCA-positive tumors were subsequently visualized by fluorescence *in situ* and *ex vivo* (see **Figure 1**).

In order to radiolabel with ^{89}Zr, a metal chelator desferrioxamine (DFO) was conjugated to A11 cMb by maleimide chemistry, or SCN-DFO was labeled to random lysines. ^{89}Zr-DFO-A11 cMb demonstrated specific tumor targeting in subcutaneous PSCA-positive tumors. In an orthotopic model, imaging with ^{89}Zr-DFO-A11 cMb-Cy5.5 resulted in a 3:1 tumor-to-blood ratio, and fluorescence clearly distinguished prostate tumor from adjacent tissues. In conclusion, the novel A11 cMb has been successfully used for visualization of tumor burden by immuno-PET and fluorescence imaging, which has the potential for clinical translation.

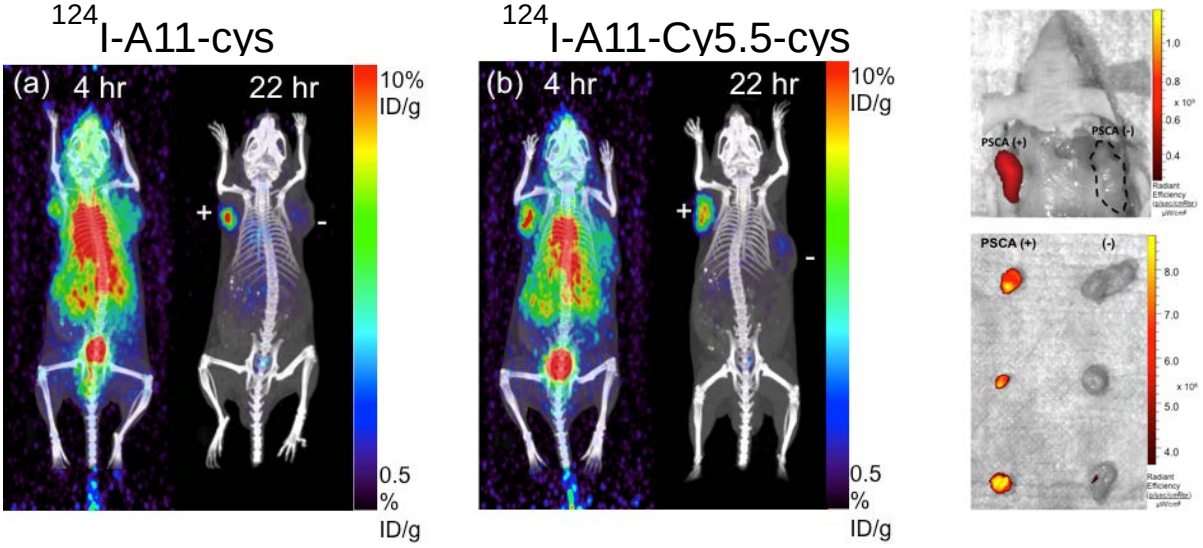


Figure 2. Dually-labeled A11 anti-PSCA cys-minibody. A11 cys-minibody was reduced using TCEP and site-specifically conjugated to maleimide Cy5.5 fluorescent dye. Non-conjugated and dye-conjugated cys-minibodies were then radiolabeled with I-124 for PET imaging. Mice bearing 22rv1 (right shoulder) and 22rv1-PSCA (left shoulder) were injected with singly- or dually-labeled A11 PSCA cys-minibodies. Serial PET imaging at 4 and 22 hrs show excellent localization of both probes to PSCA+ tumors by 22 h post injection. Mice were euthanized, and subject to fluorescent imaging following removal of skin (top right); isolated tumors were subsequently removed and also imaged optically (bottom right).

Ongoing activities include collaborative studies with Co-investigator Dr. Jennifer Murphy and her laboratory, developing site-specific F-18 radiolabeling approaches (Major Task 2, Subtask 2). We have also embarked on the development of multifunctional linkers that will carry both the PET and optical tags (Major Task 2, subtask 5). Finally, work continues on development of "click" chemistry approaches for rapid, efficient radiolabeling (Major Task 3, subtask 2). We expect to report on progress on these tasks next year.

We also attempted to develop and implement a site-specific conjugation approach based on introduction of specific mannosylation sites into the engineered proteins. (Major Task 2, Subtask 4 and Major Task 3, Subtask 1). Thus far, efficiency of mannosylation has been too low to be useful. We will continue to try to develop this approach but it may not be feasible. Regardless, as our overall strategy included parallel approaches to achieve our goals, we will still be able to achieve our

overall objectives, as demonstrated by our success with the dually-labeled cys-minibodies, described above.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

During the next year we will be able to transition our lead dually-labeled PET/optical probes to more biologically relevant models of prostate cancer including bone and lymph node metastasis models as well as genetically engineered mouse models, in close collaboration with the overall PI, Dr. Robert Reiter. We will also begin to apply these in intraoperative models of prostate cancer, again in with Dr. Reiter. Finally, we will focus on the dual F-18/optical probes (which will be based on the smaller A2 cys-diabody because its in vivo kinetics are better matched to the physical half-life of F-18).

4. IMPACT

What was the impact on the development of the principle discipline of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report. After only one year of funded research it is too early for our findings to have significant impact.

5. CHANGES/PROBLEMS

Nothing to report. There are no significant changes to the objectives, scope, and approaches of the project.

6. PRODUCTS

Publications, conference papers, and presentations

Abstracts

Tsai, Wen-ting, Tavaré, R., Zettlitz, K.A., Salazar, F.B., Knowles, S., **Reiter, R.,** and **Wu, A.M.** (2015). Dual modality immunoPET/fluorescence imaging of prostate cancer. World Molecular Imaging Congress, Honolulu, HI.

Tsai, W.-T., Tavaré, R., Zettlitz, K.A., Salazar, F.B., **Reiter, R.E.,** and **Wu, A.M**. (2015) Dual-modality immunoPET/fluorescence imaging of prostate cancer using anti-PSCA cys-minibody. Antibody Engineering and Therapeutics 2015, San Diego, CA.

Tsai, W.-T., Zettlitz, K., Tavaré, R., Salazar, F., **Reiter, R.,** and Wu, A. Dual-modality immune-PET/fluorescence imaging of prostate cancer using anti-PSCA A11 cys-minibody. (2016) Antibody Technology Resource Center Symposium, San Francisco, CA.

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Anna Wu*
Project Role:	Partnering Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Wu oversaw all aspects of work performed and accomplished to-date.*
Funding Support:	

Name:	*Jennifer Murphy*
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Murphy had performed work on the design and evaluation of the chemical and radiochemical strategies for combined PET/optical labeling.*
Funding Support:	

Name:	*Amanda Freise*
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	*2*
Contribution to Project:	*Ms. Freise had performed work on site-specific conjugation, radiolabeling, and imaging of engineered antibody fragments.*
Funding Support:	

Name:	*Christopher Waldmann*
Project Role:	Postdoctoral Researcher
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*
Contribution to Project:	*Dr. Waldmann had performed work on developing methods for F-18 labeling of engineered antibody fragments.*
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Changes in active other support of PI (Dr. Anna Wu)
The following funding has ended (reported in original grant submission):

NIH R01 CA149254 Antibody-CPG conjugates for the treatment of B cell lymphoma (Timmerman, PI)

NIH U54 CA151459 Center for Cancer Nanotechnology Excellence and Translation (Gambhir, PI)

NIH R25 CA098010 UCLA Scholars in Oncologic Molecular Imaging (Phelps, PI)

NIH R21 AI114255 In vivo imaging of T cells using engineered antibodies and PET (Wu, PI)

NIH R21 CA190044) (PQC4) Imaging CD8 T cells in tumor immunotherapy by immunoPET (Wu, PI)

The following funding has been renewed and/or is active:

Renewal funding: R01CA174294 Multifunctional immunoPET tracers for pancreatic and prostate cancer (Wu, Reiter, Multi-PIs); time commitment of 1.8 calendar months; renewal project period of 8/1/2016 - 7/31/2021

New funding: Cornell University (NSF prime) SNM: Scalable Cell-free Protein Manufacturuing via NanoClay-DNA (NanoCD) Microdonuts (Luo, PI); time commitment of 0.6 calendar months; project period of 9/15/2015 – 8/31/2019

Changes in active other support of Co-Investigator (Dr. Jennifer Murphy)
New funding: R01CA174294 Multifunctional immunoPET tracers for pancreatic and prostate cancer (Wu, Reiter, Multi-PIs); time commitment of 0.60 calendar months; project period of 8/1/2016 – 7/31/2021

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Collaborative awards

This report covers the activities of the Partner PI, Dr. Anna Wu.

9. APPENDICES

None